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The usefulness of whole genome sequencing in the management of *Staphylococcus aureus* infections

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Abstract

Staphylococcus aureus remains a leading cause of hospital-acquired and community-associated infection worldwide. The burden of disease is exacerbated by the emergence of virulent strains with reduced susceptibility to commonly used antibiotics and their dissemination in healthcare settings and in the community. Whole genome sequencing (WGS) has the potential to revolutionize our understanding and management of *S. aureus* infection. As a research tool, WGS has provided insights into the origins of antibiotic-resistant strains, the genetic basis of virulence, the emergence and spread of lineages, and the population structure of *S. aureus*. As a frontline tool, WGS offers the prospect of a method that could be used to predict resistance, assess virulence, and type isolates at the highest possible resolution. The results generated could be used to guide clinical management and infection control practice. Studies using bench-top sequencing machines have already demonstrated the feasibility of such approaches. Infection control management is compromised by our incomplete understanding of transmission, which in turn reflects the suboptimal resolution offered by conventional typing methods. As the costs of sequencing begin to approach those of conventional methods, high-resolution typing with WGS could realistically be implemented for hospital infection control, as well as for local and national surveillance practice. Translation into routine practice will require the development of a knowledge base, reliable automated bioinformatic tools, the capacity to store, exchange and interrogate large volumes of genomic data, and an acceptance of WGS by clinicians, infection control specialists, and laboratory staff.

Keywords: MRSA, *Staphylococcus aureus*, transmission, virulence, whole genome sequencing

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Introduction

Whole genome sequencing (WGS) allows comparison of the genetic differences between organisms down to the resolution of a single base pair. WGS is becoming faster and cheaper. It is already possible, with bench-top machines, to turn around sequence analysis of *Staphylococcus aureus* within days [1]. The sequence assembly of a genome of approximately 3×10^6 bp currently costs *c.* €60 ($75) with next-generation sequencing platforms. This reflects a 400-fold reduction in costs as compared with only 8 years ago [2]. It seems reasonable to predict that costs will soon match those of conventional typing techniques [3]. With increasing affordability, it is conceivable that sequence-based methods might eventually replace traditional ones, not only for reference purposes, but also for frontline diagnostics. Meanwhile, WGS is proving to be a valuable research tool that can enhance our understanding of bacterial population structure, evolution, pathogenesis, and virulence. We review some recent research, and suggest how WGS could be deployed in diagnostic laboratories (Table 1). We describe some of the hurdles that might impede deployment, and outline the advantages over conventional methods.

Alexander Ogston drew attention to '*staphylococcus*' as an agent of postsurgical sepsis more than 100 years ago [4]. *S. aureus* continues to cause life-threatening invasive disease worldwide [5,6]. Mortality rates for bacteraemia remain high,

TABLE 1. Current and realistic future uses of whole genome sequencing in the management of *Staphylococcus aureus* infection

Current uses	Potential uses
Outbreak analysis (retrospective)	Rapid species identification
Epidemiological surveillance (retrospective)	Antibiotic susceptibility testing
Genome-wide association studies	Detecting virulence determinants
Reference genomes	Epidemiological surveillance (local, national, global)
	Outbreak detection (at point of first secondary case)
	Host–pathogen relationship
	Global standard for typing isolates
	Culture-free sequencing

at 30% [7]. Prompt effective antibiotic treatment is important in order to optimize clinical outcome [8]. Hence, clinicians need reliable rapid tests to determine susceptibility. Increasing awareness of methicillin-resistant *S. aureus* (MRSA) as an agent of nosocomial infection has made disease prevention and control key targets in many countries. The control and management of *S. aureus* infections is hindered by incomplete understanding of pathogenesis and virulence, and by limitations in our ability to characterize highly related strains with sufficient resolution to inform on routes of transmission. *S. aureus* strains can be assigned to lineages (or clonal complexes (CCs)) that share characteristics because of common descent [9–11]. Conventional typing techniques such as multilocus sequence typing and *spa* typing rely on variation in one or more genes to allocate strains to lineages. The short sequences (400–4000 bp) characterized by conventional typing methods account for only a fraction of the whole genome (*c.* 2.8 million nucleotides) [12]. In suitably equipped laboratories, *spa* typing can be turned around rapidly. However, such methods must be performed as an adjunct to conventional frontline methods of identification and characterization. It may take weeks before informative results can be obtained from a reference laboratory (Fig. 1). WGS offers the prospect of a frontline method that could enable species identification and prediction of antibiotic resistance combined with high-resolution typing in a single rapid test.

Understanding and Predicting Antimicrobial Resistance

Knowledge of the genetic basis of resistance will allow the development of WGS-based approaches to antibiotic resistance testing and will aid drug discovery. As a research tool, WGS has been used to confirm previous assumptions made about mechanisms of antibiotic resistance, and has also provided novel insights. For example, sequencing technologies have confirmed that *S. aureus* can acquire methicillin resistance as a result of interspecies transfer of *mecA* from *Staphylococcus epidermidis* [13]. Similarly, sequencing technology has confirmed that vancomycin-resistant *S. aureus* strains acquired resistance genes from enterococci [14,15]. In addition, WGS has shown that individual and accumulated point mutations are also associated with reduced glycopeptide susceptibility [16–18]. Sequencing of serial isolates from patients treated with daptomycin showed that mutations in phospholipid biosynthesis pathways are associated with reduced susceptibility [19]. *S. aureus* strains can become co-resistant to daptomycin and vancomycin as a consequence of exposure to vancomycin alone. In a study of isolates collected from a patient being treated with vancomycin, WGS demonstrated the appearance of mutations coincident with the emergence of daptomycin resistance [18].

WGS could also be developed into a tool to guide clinical decision-making for the care of individual patients by interrogation of the entire genome for the presence of mobile genetic elements or point mutations known to confer resistance. This comes at almost no additional cost to the initial costs of sequencing, and potentially could be more rapid than traditional culture-based methods and more comprehensive than PCR-based methods. In addition, a genetic method could be less prone to user errors such as inaccuracies in measuring inoculum densities or zone sizes.

In the context of MRSA outbreak investigations, it has been shown that *in silico* interrogation of WGS data can reliably predict antibiotic susceptibility phenotype [1,20]. A study using a panel of 18 genes associated with resistance to ten commonly used antistaphylococcal agents yielded overall sensitivity and specificity of >95% (IDweek 2012, abstract 35530). These results are comparable with those obtained by phenotypic methods such as Vitek [21], and provide further support for the use of WGS as a first-line screening method. The sensitivity can be increased by the addition of further genes to the panel at no extra cost, and previously sequenced isolates can be screened almost instantly for newly recognized resistance genes.

However, the potential to predict phenotype from genetic information is contingent on a thorough knowledge of resistance mechanisms. Before this can occur, a robust evaluation of the relationship between the 'resistome' as determined by WGS and current reference standard phenotypic methods will be necessary, analogous to the ongoing work required to determine breakpoints used in broth dilution or disk diffusion methods. An up-to-date accessible database of resistance genes will also be needed.

It is unlikely that *in silico* prediction will entirely replace culture as a predictor of clinical response to therapy, as the